The biosynthesis of albicidin

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INTRODUCTION

Albicidin is a potent inhibitor of bacterial DNA gyrase with IC_{50} values in a nM range, produced by the sugarcane pathogenic bacterium *Xanthomonas albilineans* [1]. The structure of albicidin remained unclear for more than three decades after its first description by Birch *et al.* [2]. After the identification and sequencing of three gene islands, responsible for the albicidin biosynthesis, a PKS-NRPS hybrid, build up by three enzymes, Alb01, Alb05 and Alb09 was proposed for the albicidin assembly [3, 4]. Most recently we were able to solve the hitherto unknown structure, revealing a unique polyaromatic oligopeptide mainly composed of p-amino benzoic acids [5, 6]. *In-vitro* studies of the non-ribosomal albicidin assembly line provided further insights into the biosynthetic machinery of albicidin. Together with our bioinformatic investigations we were able to propose a comprehensive biochemical assembly, expanding the non-ribosomal code of adenylation domains with p-amino benzoic acid derivatives. Furthermore our study reveals a new type of dehydratase domain responsible for the *in situ* formation and incorporation of cyano-alanine [6].

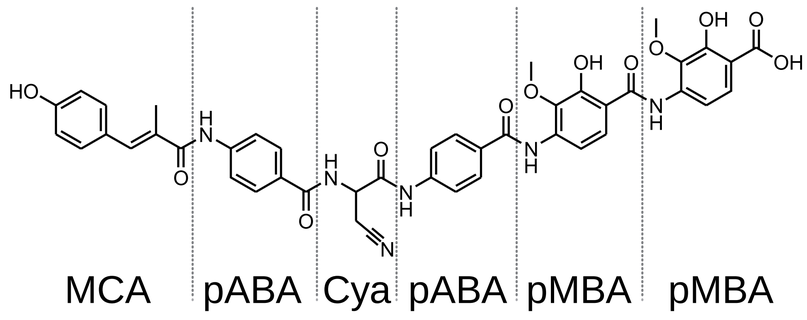

Figure 1. The structure of the albicidin. Albicidin is composed of a methylated derivative of p-coumaric acid (MCA), the non-proteinogenic α-amino acid cyanoalanine (Cya) as well as the aromatic δ-amino acids p-aminobenzoic acid (pABA, pABA) and 4-amino-2-hydroxy-3-methoxybenzoic acid (pMBA, pMBA) [5, 6].

RESULTS AND DISCUSSION

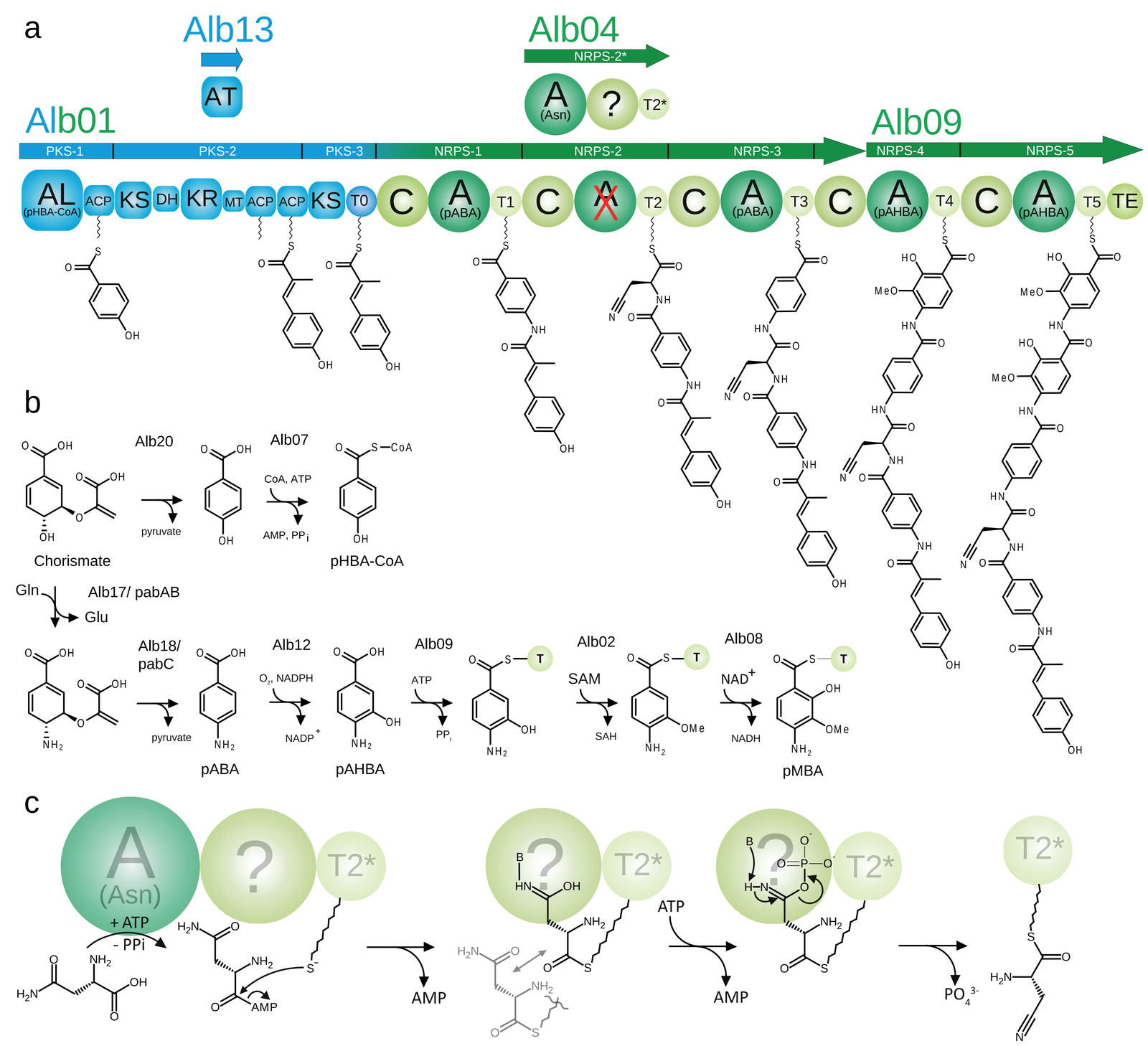

Figure 4. Model of albicidin biosynthesis. a) Proposed biosynthetic assembly line for albicidin. Substrates of the NRPS are indicated at the A domains. PKS and NRPS modules are color-coded in blue and green, respectively. b) Suggested pathways for the biosynthesis of the MCA-1 precursor pHBA-CoA as well as the δ-amino acids pABA and pMBA. c) Suggested mechanism for the transformation of activated Asn into Cya by Alb04. Conventional activation of Asn by adenylation is mediated by the A domain of Alb04. The substrate is subsequently stored as a thioester at the 4'-phosphopantetheine arm (T2* domain). A second activation occurs by phosphorylation of the Asn side-chain, which finally leads to a formal elimination of water and thus to the formation of Cya.

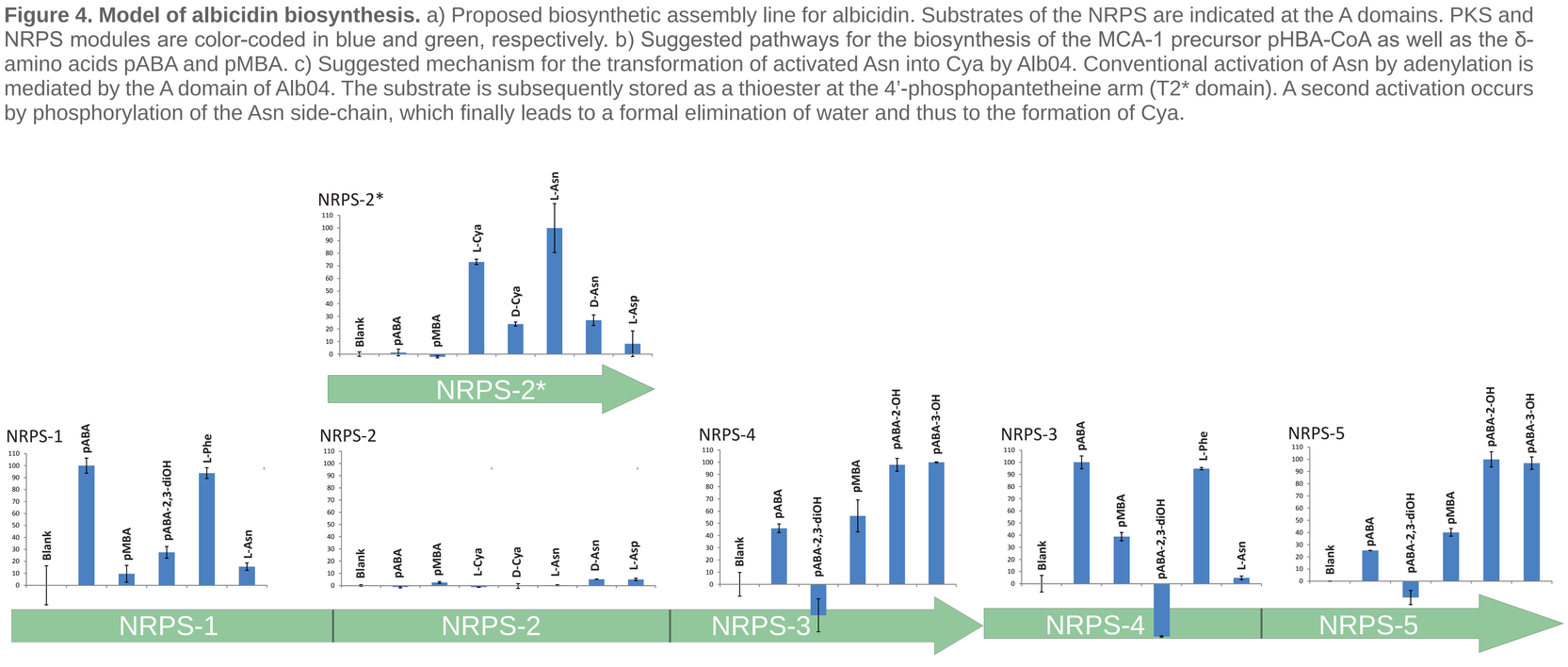

Figure 5. Substrate specificities of NRPS activation domains in albicidin biosynthesis. Relative turnover of activated substrates are shown. Radioactive ATP/PPi exchange assays were performed for the A domains of NRPS-1, NRPS-2, NRPS-2*, NRPS-3, NRPS-4 and NRPS-5. Normalization to 100% refers to 65.9 µCi/mol (NRPS-1), 1160.8 µCi/mol (NRPS2 and NRPS-2*), 168.3 µCi/mol (NRPS-3), 259.8 µCi/mol (NRPS-4) and 557,2 µCi/mol (NRPS-5), respectively. Experiments were performed in duplicates and error bars of standard deviation are shown.

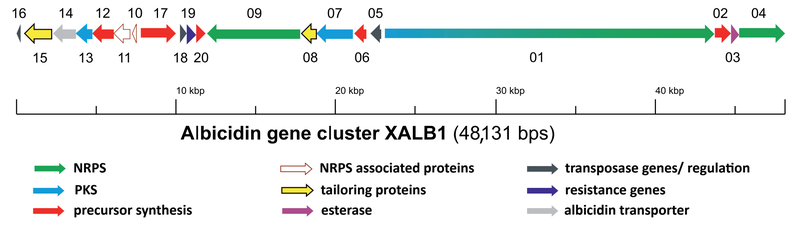

Figure 2. Gene cluster of albicidin biosynthesis. The gene machinery responsible for albicidin biosynthesis is located on three loci (XALB1-3). XALB1 contains the main genes. XALB2 and XALB3 encode each only one ORF, a phosphopantetheinyl transferase and a heat shock protein HtpG [3, 4].

a

Module	A domain signature (235 236 239 278 299 301 322 330 331 517)	Predicted substrate
GrsA	D A W T I A A I C K	
NRPS-1	A V K Y V A N D A K	NO HIT
NRPS-2	E L T Y V H A - - R	NO HIT
NRPS-2*	D L T K I G E V G K	Asx
NRPS-3	A V K Y V A N D A K	NO HIT
NRPS-4	A I K Y F S I D M K	NO HIT
NRPS-5	A I K Y F S I D M K	NO HIT

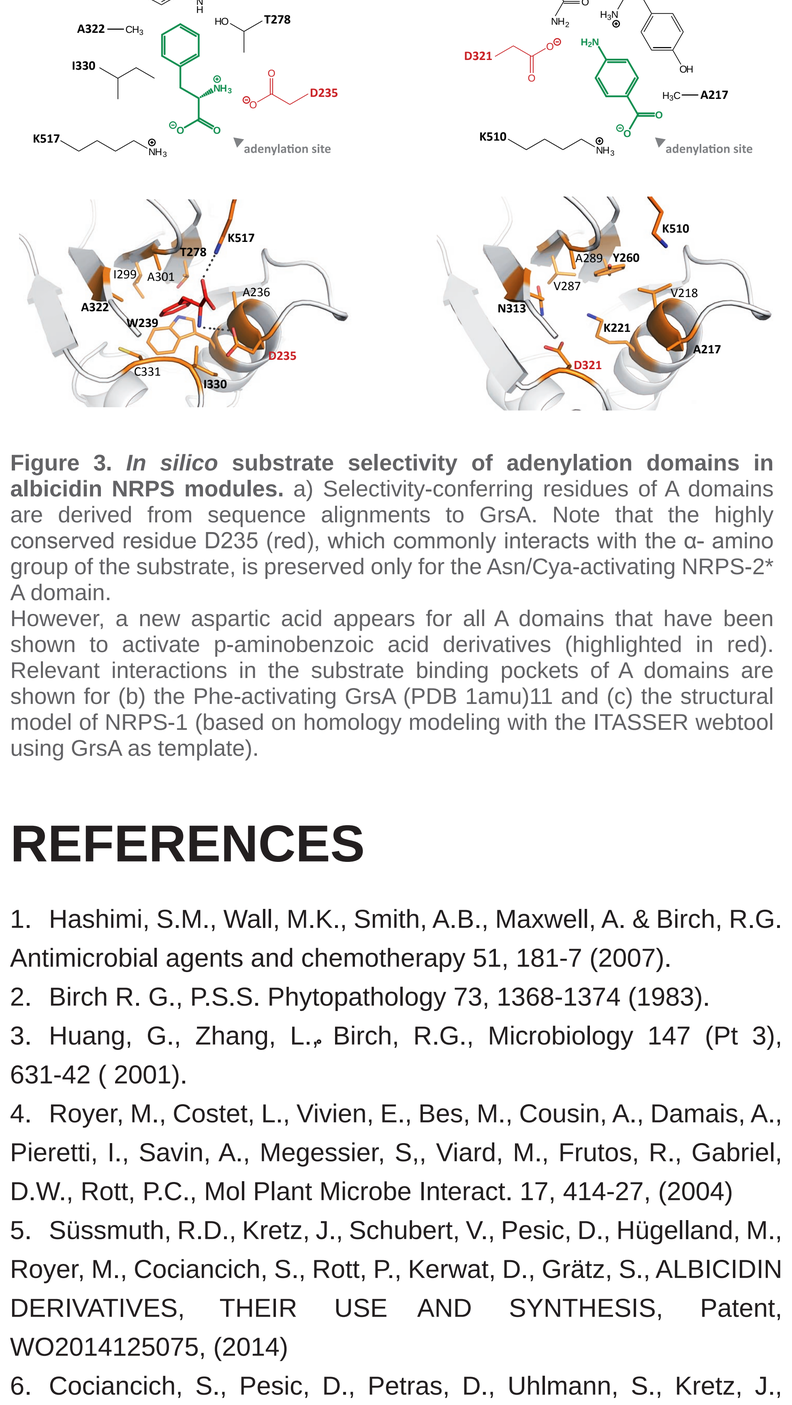

Figure 3. *In silico* substrate selectivity of adenylation domains in albicidin NRPS modules. a) Selectivity-conferring residues of A domains are derived from sequence alignments to GrsA. Note that the highly conserved residue D235 (red), which commonly interacts with the α- amino group of the substrate, is preserved only for the Asn/Cya-activating NRPS-2* A domain.
However, a new aspartic acid appears for all A domains that have been shown to activate p-aminobenzoic acid derivatives (highlighted in red). Relevant interactions in the substrate binding pockets of A domains are shown for (b) the Phe-activating GrsA (PDB 1amu)11 and (c) the structural model of NRPS-1 (based on homology modeling with the ITASSER webtool using GrsA as template).

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